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Dissociation of DNA-polycation complexes by polyanions and polyampholytes

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Abstract

Both polyanions and polyampholytes cause the dissociation of DNA-polycation complexes in experiments. To elucidate their mechanisms, Monte Carlo simulations have been conducted on a simple model, with DNA modeled as an infinite charged cylinder and other polyions treated as charged Shish-Kebab rods. Our results show that a highly charged polyanion is required to separate a polycation from a DNA. However, for a diblocked polyampholyte, its net dipole induces a higher probability to bridge a DNA and a polycation. Thus, the loosening mechanisms are found to be markedly different between polyanions and polyampholytes.

Key words: Computer Simulations, Polyelectrolytes, Polyampholytes, DNA

1 Introduction

Gene transfer is a frontier research topic to combine the effort from numerous areas of science. To facilitate gene delivery across biological cells, it is common to stabilize a negatively charged DNA with polycations that prevent genes from enzyme degradation. However, some highly stable DNA-polycation complexes impede transcript factors to access DNA and reduce the mRNA transcript production, a crucial step for gene activities in vivo. The technical problem lies in how the tightly bound DNA-polycation complexes can be relaxed for transcription after transferred across cytoplasmic membranes, while the DNA stability is not compromised. Recently, Koyama and coworkers have found that certain polyanions partially loosen up DNA-polycation complexes without dissembling the complex entity completely. As such, those polyanions enhance the transcriptional activity of DNA-polycation complexes. [1,2]

In addition to the above polyanions, their amphoteric derivatives have also been synthesized, containing both positively charged amino groups and negatively charged carboxylic groups. [1,2] As a matter of fact, these polyampholytes can exhibit a even greater effectiveness on the transcriptional activity compared to original polyanions. To better understand the fundamental difference between polyanions and polyampholytes on DNA-polycation complexation, theoretical studies are greatly needed.

Complexation of ionic polymers has been addressed from theoretical standpoint. [3–5] Kunze et al investigated a flexible polyelectrolyte chain near an oppositely charged rod. The adsorbed flexible chain takes the rodlike and

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helical conformation at high and low salt condition, respectively. [3] Moreover, Jeon and Dobrynin have conducted simulations to examine the binary polyampholyte-polyelectrolyte complexes. [6] They found that in contrast to the free chain, a polyampholyte tends to elongate while it undergoes complexation with a rigid polyelectrolyte. Moreover, the binding strength of binary complexes is sensitive to the polyampholyte sequence. A random polyampholyte binds with the polyelectrolyte more strongly than an alternating polyampholyte due to more dipolar interactions in random polyampholytes.

Here, a simplified model is devised to shed light on the essential properties of the two ternary systems: DNA/polycation/polyanion and DNA/polycation/polyampholyte. Our primary focus is to understand the subtle difference between polyanions and polyampholytes on the polycation-DNA dissociation. For simplicity, electrostatic potentials are considered at the Debye-Hückel level, without explicit small ions incorporated. Meanwhile, DNA is modeled as an infinite charged cylinder, similar to the cell model, and all other ionic polymers are treated as rigid Shish-Kebab chains. [7] The detailed fluorescence images by Kidoaki and Yoshikawa have disclosed that the stiff DNA conformation, i.e., the rigid double-stranded structure, remains after binding with polycations. [8] Namely, this simplified model can be a reasonable approximation to depict the local structure of a DNA/polyion complex. Moreover, Shish-Kebab chains reflect the theoretical predictions of the binary polycation-DNA [3] and polyampholyte-rigid polyelectrolyte complexes [6], and manifest the elongated conformation of ionic polymers induced by adsorption. The rigid chain model enables us to investigate the structure of ternary systems, as a reference point prior to the study of more complex flexible chain systems. The essence of this work is to explain the experimental findings through fundamental physics. Our calcula-

tions are to link the general features induced by electrostatic interaction with the experimental observation.

2 Experiment

In the experiment, a plasmid DNA/polyethyleneimine (PEI) complex was prepared by mixing their solutions, where PEI is a typical polycation. This complex was then mixed with the poly(ehylene glycol) (PEG) derivative with carboxylic acid side chains (PEG-C; Mn=8940, 17.7 COOH groups per molecule) or the PEG derivate with both amino and carboxylic acid side chains (PEG-AC; Mn=8750, 6.5 NH_2- and 11.2 $COOH$-groups per molecule). The synthetic procedures and the detailed properties of anionic PEG-C and amphoteric PEG-AC can be found elsewhere. [1,9] The ternary complex DNA/PEI/PEG-C (or PEG-AC) was prepared at feed ratio 1 : 1 : 24 in weight to compare with the control experiment containing the binary mixture of DNA and PEI (1 : 1 by weight). The transcriptional activity was tested with RNA polymerase (from *E. coli*) in vitro, by measuring the increase of fluorescence intensity due to the conversion of γ-AmNS-UTP into AmNS and UTP during the polymerase process. [1]

3 Model and Simulation

A model, similar to the cell model [7], is devised to elucidate the dissociation of a DNA-polycation complex mediated by a polyanion or a polyampholyte, as shown in Figure 1. DNA is modeled as an infinite charged cylinder, of which charge density and radius ($R_{DNA} = 10\mathring{A}$) are set equal to those of DNA. In

order to reduce the computational time without loosing the basic physico-chemical characteristics on the DNA complex, we adapt the approximation that the charged cylinder is surrounded by a concentric cylindrical and impenetrable simulation cell of which radius (R_{cell}/R_{DNA}) is chosen to be 12 and cell length L is $18R_{DNA}$. These ionic polymers are modeled as rigid Shish-Kebab chains containing six tangent hard spheres (i.e., monomers) aligned along the same axis. The diameter of charged monomers σ (with numerous charged groups) is set equal to the DNA radius (σ=R_{DNA}). The magnitude of a monomer charge is denoted as f_+ for polycation, f_- for polyanion, and $f_\pm$ for polyampholyte. To test the effect of chain architectures, alternating and diblocked polyampholytes, with zero net charge, are investigated.

The interaction potentials considered in the model include hard core repulsions and electrostatic interactions. The hard core repulsions are given by

$$\begin{aligned} V_{ex}(r_{ij}) &= 0 \qquad if \;\; r_{ij} > \sigma_{ij} \\ &= +\infty \quad otherwise \end{aligned} \tag{1}$$

where r_{ij} is the distance between two intermolecular charged monomers of species i and j, and σ_{ij} is the cut-off distance for the corresponding hard core repulsion. For DNA, $r_{DNA,j}$ is the radial distance of a charged monomer of species j from the DNA axis, and $\sigma_{DNA,j} = 1.5R_{DNA}$. For polycations and polyanions (or polyampholytes), r_{ij} is the separation between two monomers of polymer species i and j, and $\sigma_{ij} = \sigma$.

Here, electrostatic interactions are considered at the level of Debye-Hückel theory. The smaller ions are collapsed into the Debye screening length κ^{-1}, and solvents are treated as a dielectric continuum. With these simplifications, the investigated system is reduced to a ternary system, and the effect of coun-

terion condensation can be incorporated as suggested by Manning [10] and by Stigter [11]. After counterion condensation is taken into account, the Debye-Hückel potential between the infinite charged cylinder and the surface of the concentric cylindrical simulation cell reads [10,12]

$$V(r)/k_BT = (\frac{2}{\kappa R_{DNA}})\frac{K_0(\kappa r)I_1(\kappa R_{cell}) + I_0(\kappa r)K_1(\kappa R_{cell})}{K_1(\kappa R_{DNA})I_1(\kappa R_{cell}) - I_1(\kappa R_{DNA})K_1(\kappa R_{cell})} \quad (2)$$

where I_0 and I_1 are the modified Bessel functions of the first kind; K_0 and K_1 are the modified Bessel functions of the second kind; k_B is the Boltzmann constant; T is the temperature (room temperature in this work). Note that in the simulations, κ is chosen to be $0.8/R_{DNA}$, equivalent to the monovalent salt concentration around 0.06 M. In fact, equation (2) is similar to the counterion condensation model (CC1) in which the layer of counterion condensation is assumed to be very small. [10]

In the cylindrical simulation cell, the periodical boundary condition is set along the axis, and the one-dimensional Ewald summation is chosen to compute long-ranged interactions. In fact, the one-dimensional Ewald potential for the screened Coulomb potential (or Yukawa potential) becomes a function of two variables in the cylindrical coordinate: the radial distance (r) and the distance of any two particles projected onto the cylindrical axis (z) [13], which reads

$$V(r,z) = \sum_{k=-M_1}^{M_1} \frac{\exp -[\kappa\sqrt{r^2+(z+kL)^2}]}{\sqrt{r^2+(z+kL)^2}} \quad (3)$$

$$= \frac{4}{L}\sum_{l=1}^{M_2} K_0(\frac{2\pi lr}{L})\cos(\frac{2\pi lz}{L}) - \frac{2}{L}\ln(\frac{r}{2L}) - \frac{2}{L}\ln(\kappa L) - \frac{2}{L}\gamma \quad (4)$$

where γ is the Euler constant. The first equation in $V(r,z)$ is needed for smaller r because the second equation becomes divergent in that regime. In the calculations, the first equation is used when $r/R_{DNA} < 0.6$, with $M_1 = 5$

and $M_2 = 100$. These two equations are similar to the MMM1D potential for one-dimensional Coulomb systems (consisting of short- and long-distance formulas), except that the short-distance formulation is replaced by equation (3). To expedite the calculations, the two-variable Ewald potential is tabulated in advance to alleviate computational time.

In the simulations, the configurations of ionic polymers are sampled through two types of moves: random walk and random rotation against their center-of-mass. The move is accepted by using the Metropolis acceptance criterion. [14] The length of a simulation consists of 2×10^8 moves, and a total of 1×10^7 equilibriated configurations are used to compute the equilibrium local monomer density of ionic polymers around the DNA and other properties.

4 Results and discussion

Figure 2 compares the relative transcriptional activity of the plasmid complexes. The findings indicate that both ternary complexes DNA/PEI/PEG-C and DNA/PEI/PEG-AC enhance the transcriptional activity compared to the control experiment, with simple combination between a DNA and a polycation, PEI. In Figure 2, the DNA/PEI/PEG-AC complex exhibits a higher enhancing efficiency than the DNA/PEI/PEG-C complex, suggesting polyampholytes can be more effective than polyanions. It is reported that in cell nuclei, the amphoteric HMG proteins stimulate the gene transcription via specific interactions with chromatin. [15,16] Since the PEG-AC has no such specific interactions as HMG, our results suggest that non-specific interactions may be responsible for its higher enhancing effect. [1] To elucidate the dissociation mechanisms of ternary complexes, in the following, the investiga-

tion is conducted through the simplified model based on non-specific Coulomb interactions.

We first study the mixture of a long chain DNA molecule and a polycation mixing with a polyampholyte (or a polyanion) by assuming that the monomer charge in a polycation f_+ is adjustable but the monomer charge in a polyampholyte (or a polyanion) is fixed ($f_\pm = f_- = 1$). Figure 3 plots the local monomer density $C_m(r)$ of a polycation (solid lines) and of a diblocked polyampholyte (dotted lines) for $f_+ = 0.1$, 0.3, and 0.5 in the mixture of DNA/polycation/diblocked polyampholyte; for comparison, the $C_m(r)$ of different polyions, as marked, are also plotted for the DNA-polycation mixtures in the presence of an alternating polyampholyte and a polyanion for $f_+ = 0.3$. In the range of our study, the $C_m(r)$ of a polycation is sensitive to its monomer charge f_+, but is insensitive to (low) $f_\pm$ and f_-, and the identity of the third-component polyions. Note that the $C_m(r)$ of the third-component polyions are also insensitive to the charge of polycations in Figure 3. As f_+ is increased, the probability of finding a polycation near the DNA increases due to an increase of attractive interaction.

Compared to the diblocked polyampholyte cases in Figure 3, the negatively charged polyanion exhibits the lowest $C_m(r)$, especially near the like-charged DNA. Like a polyanion, the $C_m(r)$ of an alternating polyampholyte remains repulsive to the DNA (monotonically increasing in Figure 3) except that its $C_m(r)$ becomes larger. In contrast, the behavior of a diblocked polyampholyte is different from its alternating counterpart. The corresponding $C_m(r)$ decreases first, and then increases again near the DNA, suggesting that the diblocked polyampholyte is effectively attractive to the DNA. Moreover, the effective attraction between a diblocked polyampholyte and a DNA can be

stronger than that on those lower charged polycations, e.g., $f_+ = 0.1$ and 0.3.

To investigate the DNA-polycation complex dissociation induced by a polyanion, the calculation is carried out by changing the total charge of the polyanion. Figure 4 displays the $C_m(r)$ of a polycation and a polyanion, as marked, around the DNA in the DNA/polycation/polyanion mixture for $f_- = 0$ and 1.5, as marked, when $f_+ = 1$; for comparison, we also plot the $C_m(r)$ of a polycation and a diblocked polyampholyte in the DNA/polycation/diblocked polyampholyte mixture (dash-dotted lines) for $f_+ = 1$ and $f_\pm = 1.5$. The calculation shows that when the f_- of the polyanion is increased from 0 to 1.5, both polycation and polyanion distribution near the DNA decrease. From the $C_m(r)$ of the two different polyaions ($f_- = 0$ and 1.5), one can argue that the greater charged polyanion (i.e., $f_- = 1.5$) is strongly repelled by the DNA, and tends to pull the polycation away from the DNA. Namely, a highly charged polyanion enhances the dissociation of DNA-polycation complexes. In the highly charged diblocked polyampholyte case ($f_\pm = 1.5$), the polycation distribution in the mixture decreases insignificantly (with a similar polycation denisty density distribution as that of f_-=0) compared to the ternary mixture containing a highly charged polyanion, suggesting a different dissociation pathway induced by a diblocked polyampholyte.

To discern the distinct loosening mechanisms between a diblocked polyampholyte and a polyanion, we calculate the distribution function $P(\cos\theta_c)$ where θ_c is the angle between the following two vectors: one is from the center-of-mass of a polyampholyte (or a polyanion) to that of a polycation, and the other is from the center-of-mass of a polyampholyte (or a polyanion) to the molecular axis of a DNA, as defined in Figure 5. When the polyampholyte (or the polyanion) is aligned between the DNA and the polycation, the an-

ticipated θ_c would approach π, whereas when the DNA or the polycation is situated in the middle between the other two species, θ_c would shift to 0.

Figure 5 displays the angular distribution function $P(\theta_c)$ in the range of $-1 < \cos(\theta_c) < 0.4$ for the polycation-DNA mixture in the presence of a polyanion, an alternating polyampholyte, and a diblocked polyampholyte, as marked, for f_- (and $f_\pm$) $= 1$ (dotted lines) and 1.5 (solid lines) when $f_+ = 1$. Our results show that for f_- (and $f_\pm$) $= 1$, the probability of finding a polyion at around $\cos\theta_c = -1$ (or $\theta_c = \pi$) increases in an increasing order of polyanion, alternating polyampholyte and diblocked polyampholyte. This trend is an indication that the diblocked polyampholyte exhibits the most pronounced effect on bridging a DNA and a polycation. Such a bridging mechanism becomes weak in the polyanion case because the polyanion is repelled from the like-charged DNA, and the polycation is located preferentially between the two oppositely charged polyions. As for an alternating polyampholyte, the distribution function is somehow in-between those of polyanion and diblocked polyampholyte due to its weak dipolar interaction. As $f_\pm$ and f_- are increasd from 1 to 1.5, the distribution of an alternating polyampholyte shows little change, but the distribution of a polyanion increases slightly near $\cos(\theta_c) = -1$ which may be attributed to some extent of complexation between a polyanion and a polycation. For the diblocked polyampholyte, the distribution function increases significantly because of its strong dipolar interaction. Note that the electrostatic bridging effect is enhanced further as κ is decreased (data not shown). From these findings, one may argue that polyampholyte architectures play the role to alter the strength of electrostatic bridging. In our view, electrostatic bridging may enhance the transcriptional activity by creating more space between a DNA and a polycation in such a way that transcript factors

can access DNA more frequently.

5 Conclusions

Monte Carlo simulations have been conducted to elucidate the loosening mechanisms of a polycaion-DNA complex induced by a polyanions or a polyampholyte. The simulation cell consists of a cylindrical cell with an infinite charged cylinder to model a long DNA chain, and polyions are treated as Shish-Kebab chains. The charged particles interact through hard core repulsions and Debye-Hückel potentials. The one-dimensional Ewald summation is used to compute long-ranged interactions. We find that a polyanion exhibits the lowest probability to distribute around the like-charged DNA. To loosen up a polycation-DNA complex through a polyanion, a highly charged polyanion is required. While the diblocked polyampholyte is introduced, the loosening process is undertaken by a different mechanism. The diblocked polyampholyte induces an effective attraction with DNA; as a result, it may bridge a DNA and a polycation. Such a result may be relevant to the high transcription activity of the DNA-polycation complexes in the presence of polyampholytes. The random polyampholytes used in experiments may contain a net dipolar interaction as those in a diblocked polyampholyte to facilitate the dissociation of DNA-polycation complexes.

The experiment in this work was conducted under the good solvent condition. As such, single flexible polyelectrolytes (e.g, polycations and polyanions) should adopt the morphology between random coil and rigid rod. It has been shown that flexible polyelectrolytes exhibit slightly more pronounced electrostatic interactions because the charges on a flexible chain are less dispersed

than their rigid rod counter part. [17] In contrast to rigid rods, flexible diblocked polyampholytes may form more contract morphology due to the attraction between two oppositely charged blocks. [5,18] Nevertheless, dipole moment is expected to remain in the chain molecule, but the magnitude of dipole is anticipated to be smaller than that of rigid chains due to a decrease of the average distance between oppositely charged groups. Namely, the chain flexibility may increase and decrease the electrostatic effect on polyelectrolytes and diblocked polyampholytes, respectively. These speculations elicit an important question for the future work regarding how to design a flexible polyampholyte molecule to maximize its effectiveness in the dissociation of DNA/polycation complexes. The above issues can be tested in the future simulations together with incorporating explicit counterions and coions.

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Fig. 1. Schematic plot of the simulation model consisting of DNA, polycation and polyampholyte.

Fig. 2. Comparison of the relative transcriptional activity of the plasmid and its complexes. The plasmid complexes were incubated with ATP, CTP, GTP, γ-AmNS-UTP, and E. Coli RNA polymerase at 37^o for 90 minutes.

Fig. 3. Plots of the local monomer density $C_m(r)$ of a polycation (solid lines) and of a diblocked polyampholyte (dotted lines) for $f_+ = 0.1$, 0.3, and 0.5 in the mixture of DNA/polycation/diblocked polyampholyte when $f_\pm = 1$; for comparison, the $C_m(r)$ of different polyions, as marked, are also plotted for the DNA/polycation mixture mixing with an alternating polyampholyte and with a polyanion, respectively, for $f_+ = 0.3$ when $f_\pm = 1$ and $f_- = 1$.

Fig. 4. Plots of the $C_m(r)$ of a polycation and a polyanion, as marked, around the DNA in the DNA/polycation/polyanion mixture when $f_- = 0$ (dotted lines) and 1.5 (solid lines), and $f_+ = 1$; for comparison, we also plot the $C_m(r)$ of a polycation and a diblocked polyampholyte in the DNA/polycation/diblocked polyampholyte mixture (dash-dotted lines) for $f_+ = 1$ and $f_\pm = 1.5$.

Fig. 5. The angular distribution function $P(\theta_c)$ in the range $-1 < \cos(\theta_c) < 0.4$ for the polycation-DNA mixture in the presence of a polyanion, an alternating polyampholyte, and a diblocked polyampholyte, as marked, for $f_\pm$ or f_- =1 (dotted lines) and 1.5 (solid lines) when $f_+ = 1$, where θ_c is defined in Figure 1.

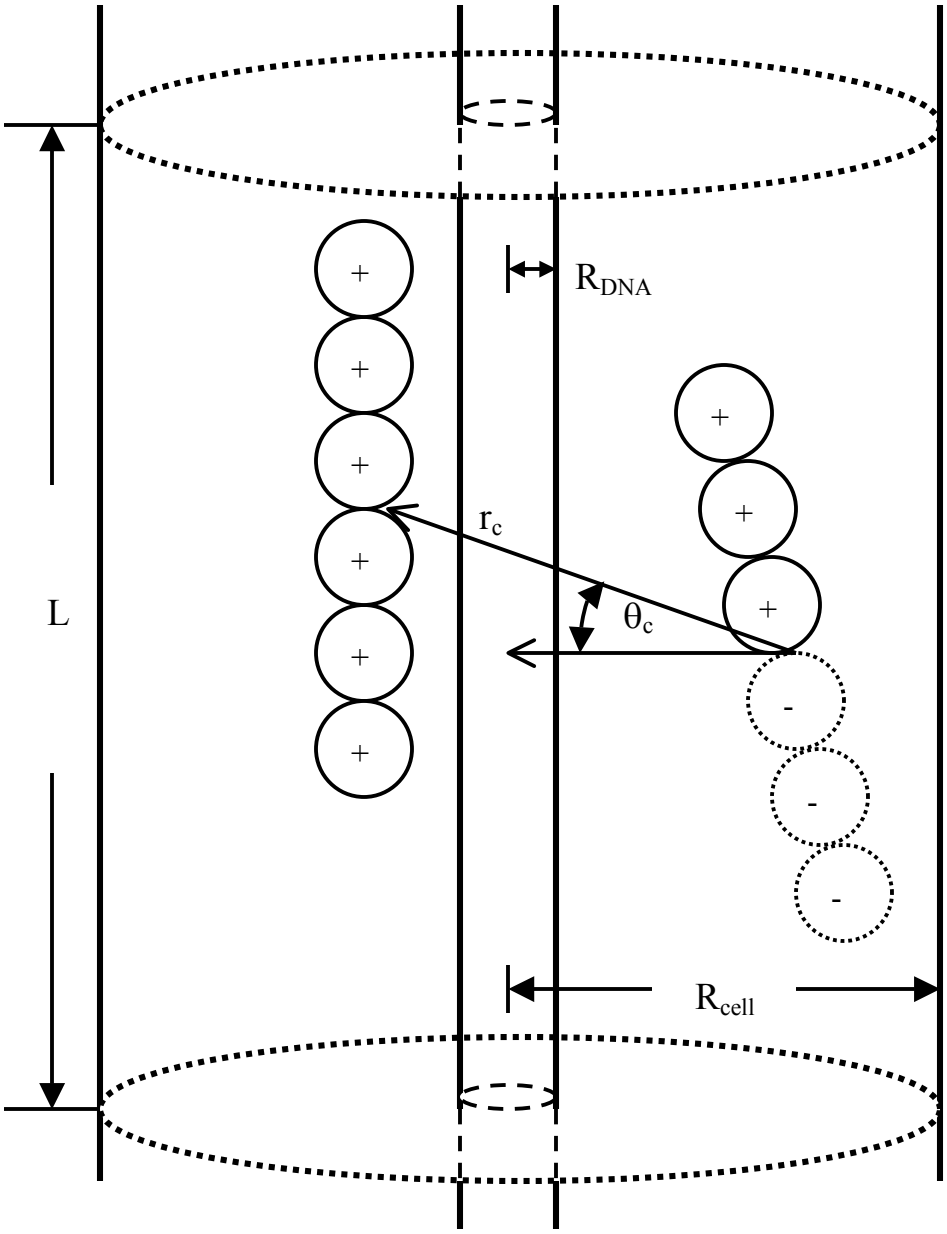


Figure 1, Shew et al

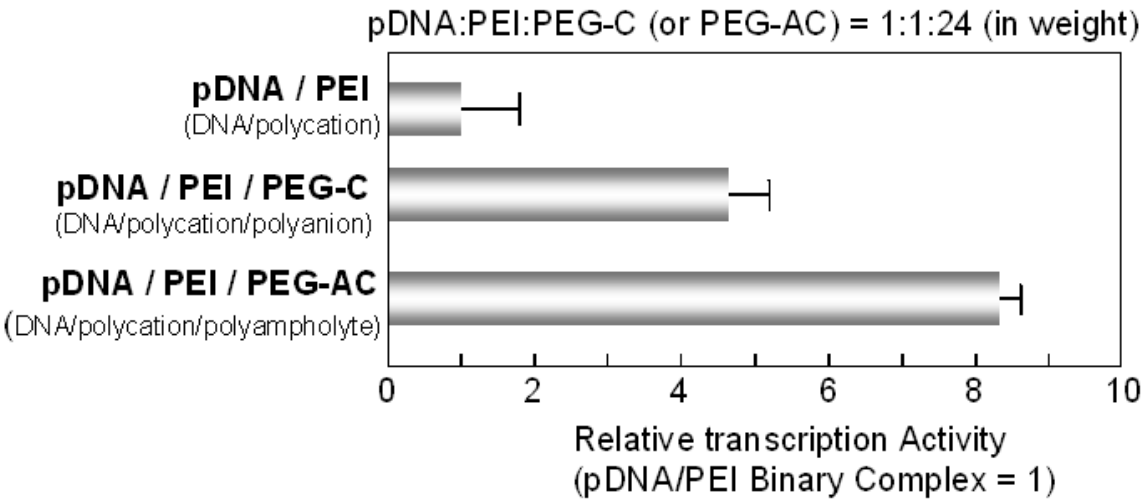


Figure 2, Shew et al

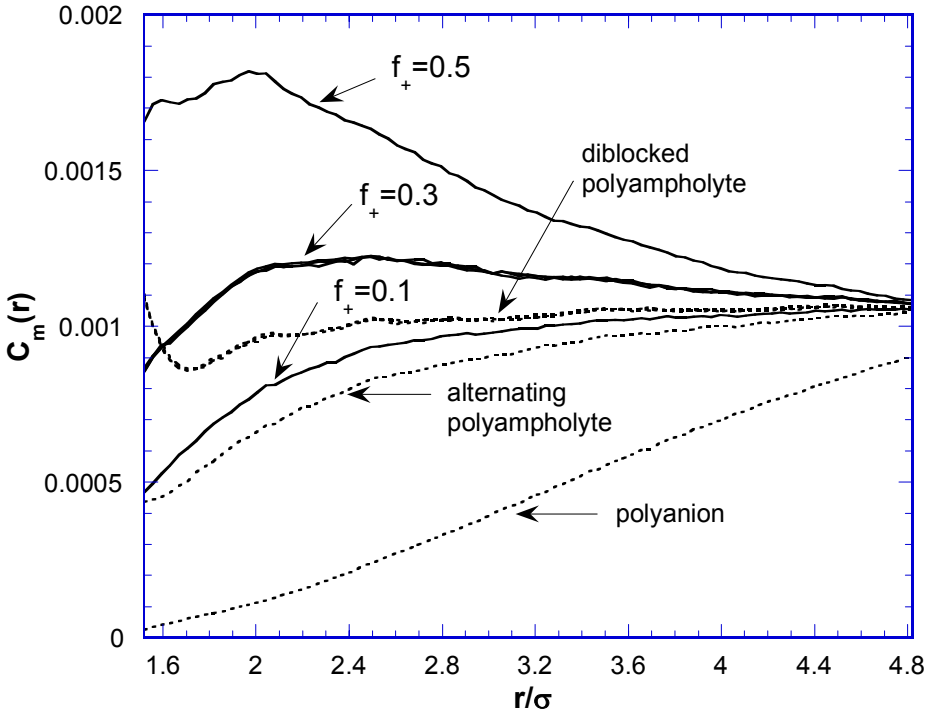
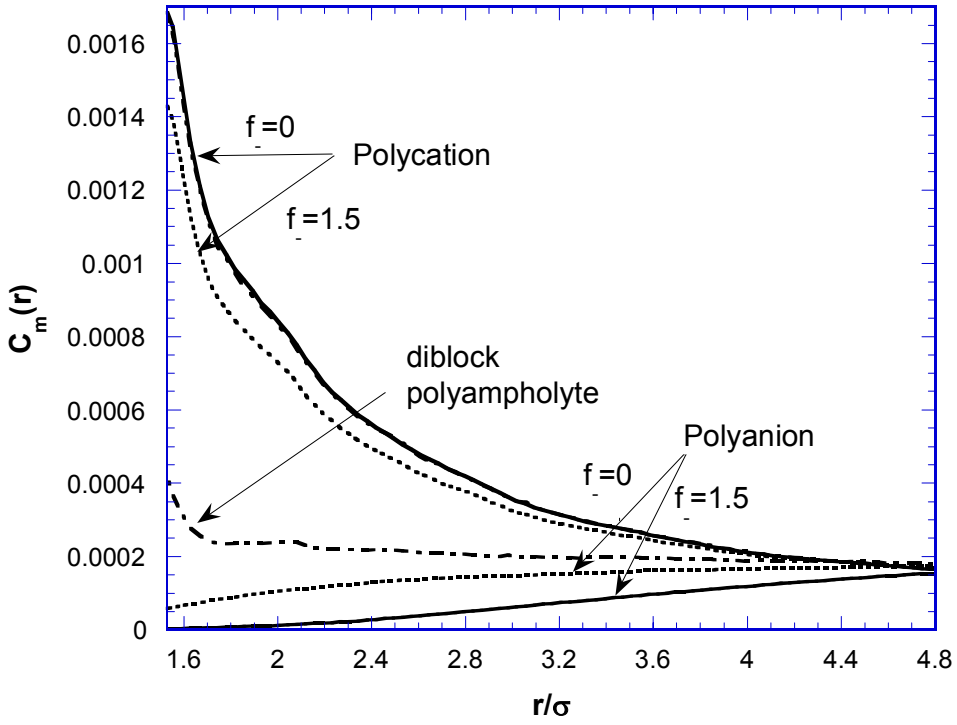


Figure 3, Shew et al



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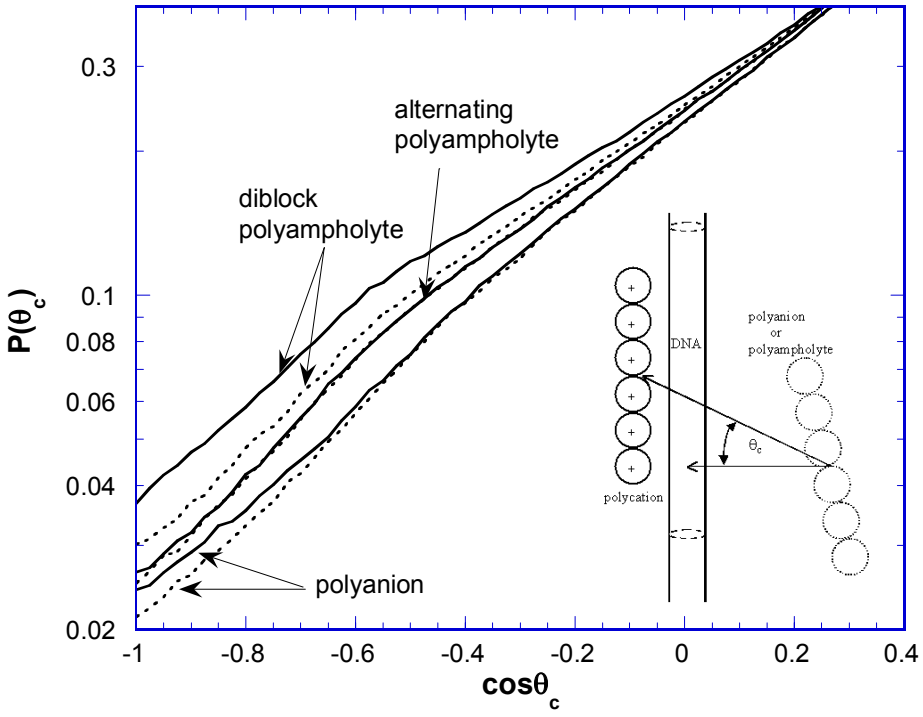

Figure 5, Shew et al